Genomics 100 (2012) 18–26

Contents lists available at SciVerse ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Global transcriptional analysis of psoriatic skin and blood confirms known disease-associated pathways and highlights novel genomic "hot spots" for differentially expressed genes

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ARTICLE INFO

Article history:
Received 26 February 2012
Accepted 6 May 2012
Available online 11 May 2012

Keywords:
Psoriasis
Microarrays
Pathogenesis
Global gene expression
PSORS loci

ABSTRACT

There are major gaps in our knowledge regarding the exact mechanisms and genetic basis of psoriasis. To investigate the pathogenesis of psoriasis, gene expression in 10 skin (5 lesional, 5 nonlesional) and 11 blood (6 psoriatic, 5 nonpsoriatic) samples were examined using Affymetrix HG-U95A microarrays. We detected 535 (425 upregulated, 110 downregulated) DEGs in lesional skin at 1% false discovery rate (FDR). Combining nine microarray studies comparing lesional and nonlesional psoriatic skin, 34.5% of dysregulated genes were overlapped in multiple studies. We further identified 20 skin and 2 blood associated transcriptional "hot spots" at specified genomic locations. At 5% FDR, 11.8% skin and 10.4% blood DEGs in our study mapped to one of the 12 PSORS loci. DEGs that overlap with PSORS loci may offer prioritized targets for downstream genetic fine mapping studies. Novel DEG "hot spots" may provide new targets for defining susceptibility loci in future studies.

1. Introduction

Psoriasis is a chronic systemic inflammatory disorder involving the skin, nails and occasionally the joints that affects approximately 2% of the Caucasian population [1,2]. The most characteristic feature of psoriasis is the thick scaly plaque resulting from the hyperproliferation of keratinocytes. Investigations over the last two decades have described many key points in disease pathogenesis and recent findings have emphasized the roles of Th1 and Th17 cells along with dendritic cells in the complex interplay between the immune system and keratinocyte activity that underlies the hyperproliferation [1–3].

The etiology of psoriasis is complex, involving both environmental and genetic factors. Linkage and association studies have reported nearly 40 susceptibility loci for psoriasis, 12 of which (PSORS1-12) have been confirmed by multiple studies. However, a comprehensive list and exact locations of susceptibility genes that would account for the development of psoriasis has not been generated, and there remain several gaps in our knowledge regarding the mechanisms by which putative susceptibility genes interact with one another and with environmental factors to initiate plaque formation.

Microarray methods have been employed frequently over the past decade by several groups to explore the psoriatic lesional 'transcriptome' [4–13]. There have also been microarray studies exploring in vivo and in vitro treatment effects on gene expression in the blood of subjects with psoriasis and a recent publication compared the gene expression in psoriatic blood to blood from healthy subjects. Chen et al. have recently performed an analysis on the results of microarray studies and shown that the differentially expressed genes (DEGs) were more likely than constantly expressed genes to be associated with disease. They have also reported that the chances of a gene to be associated with disease increased exponentially as the differential expression ratio (number of studies reporting it a gene as a DEG divided by the number of studies measuring the gene) increased and proposed the utilization of gene expression data to prioritize target candidate genes or single nucleotide polymorphisms in genome wide association studies [14].

In this study, we investigated the differential gene expression between lesional and nonlesional psoriatic skin samples to identify disease associated dysregulated pathways at the local tissue level. We also explored the degree of consensus among published microarray gene expression studies and our data to illuminate significant signatures not evident in the analysis of individual datasets. The group of DEGs shared across multiple studies highlights key genes and pathways of dysregulation that are consistent with the pathophysiologic changes taking place in the psoriatic plaque. Specifically, replicated DEGs reinforce the importance of dysregulation in 1) epidermal differentiation, 2) immune response, and 3) hypermetabolic processes in plaque formation. We also examined the gene expression in the blood of psoriatic subjects with blood from nonpsoriatics to define the disease-associated signatures at the systemic level. In psoriatic

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doi:10.1016/j.ygeno.2012.05.004

blood, we detected several modulations of gene expression associated with pathophysiologic pathways previously implicated in psoriasis, including immune response, proteolysis, apoptosis and oxidation–reduction. We also evaluated the distribution of our psoriatic skin and blood associated DEGs, along with the DEGs commonly reported in previous microarray studies on the genome to investigate their overlap with the PSORS loci. We further explored the chromosomal distribution of the genes in these three sets to detect transcriptional "hot spot" regions where DEGs were found to map significantly more frequently than should occur by chance. The "hot spots" we detected for the skin and blood DEGs found in our study and the set of DEGs replicated across microarray studies, their overlaps with each other and the PSORS loci could be expected to facilitate the pinpointing of disease-associated genes. The novel "hot spots" detected in our study may suggest previously unidentified chromosomal regions that harbor additional psoriasis susceptibility genes.

2. Results

2.1. Unsupervised clustering distinguishes lesional from nonlesional psoriatic skin

We initially analyzed the mRNA expression in 5 pairs of lesional and nonlesional skin biopsies from subjects with chronic plaque psoriasis. After low-level analysis and normalization, 3832 probes fulfilled the filtering criteria of coefficient of variation ≥ 0.3 and present call ≥ 30%. Unsupervised clustering (dChip) resulted in distinct separation of lesional and nonlesional skin samples (Fig. 1). The upregulated genes were associated with 93 gene ontology (GO) terms, including metabolic and catabolic activities, cell cycle processes, cutaneous differentiation and apoptosis (Supplemental File 1).

2.2. Evaluation of DEGs in skin supports known pathological changes associated with psoriasis

We identified DEGs in the skin by comparing the log-transformed expression indices of lesional and nonlesional samples with a paired t-test ($p \leq 0.01$) and a false discovery rate (FDR) was controlled at 1–5% levels using the Benjamini–Hochberg procedure. At 1% FDR we identified 535 DEGs in the skin, 425 of which were upregulated and 110 which were downregulated (Supplemental File 2).

From the list of differentially expressed genes, *TGM, POLE3, IVL, FABP5, IL1RN, LYN, GARS* and *ALOX12B* were randomly selected for evaluation by QRT-PCR in order to validate the results of the microarray analysis. QRT-PCR results uniformly confirmed the dysregulation of the genes relative to nonlesional skin found by microarray (Supplemental File 3).

When we analyzed the 425 upregulated genes in the skin for GO terms, the highest odds ratios were detected for terms associated with the hyperproliferative environment of the psoriatic lesion, which can be grouped in three major categories: 1) cutaneous differentiation, 2) cell cycle, and 3) metabolic processes. For the 111 downregulated genes, there were 8 significantly enriched GO terms, including oxygen binding, channel activities, heme and tetrapyrrole binding, nervous system development and transducer activities.

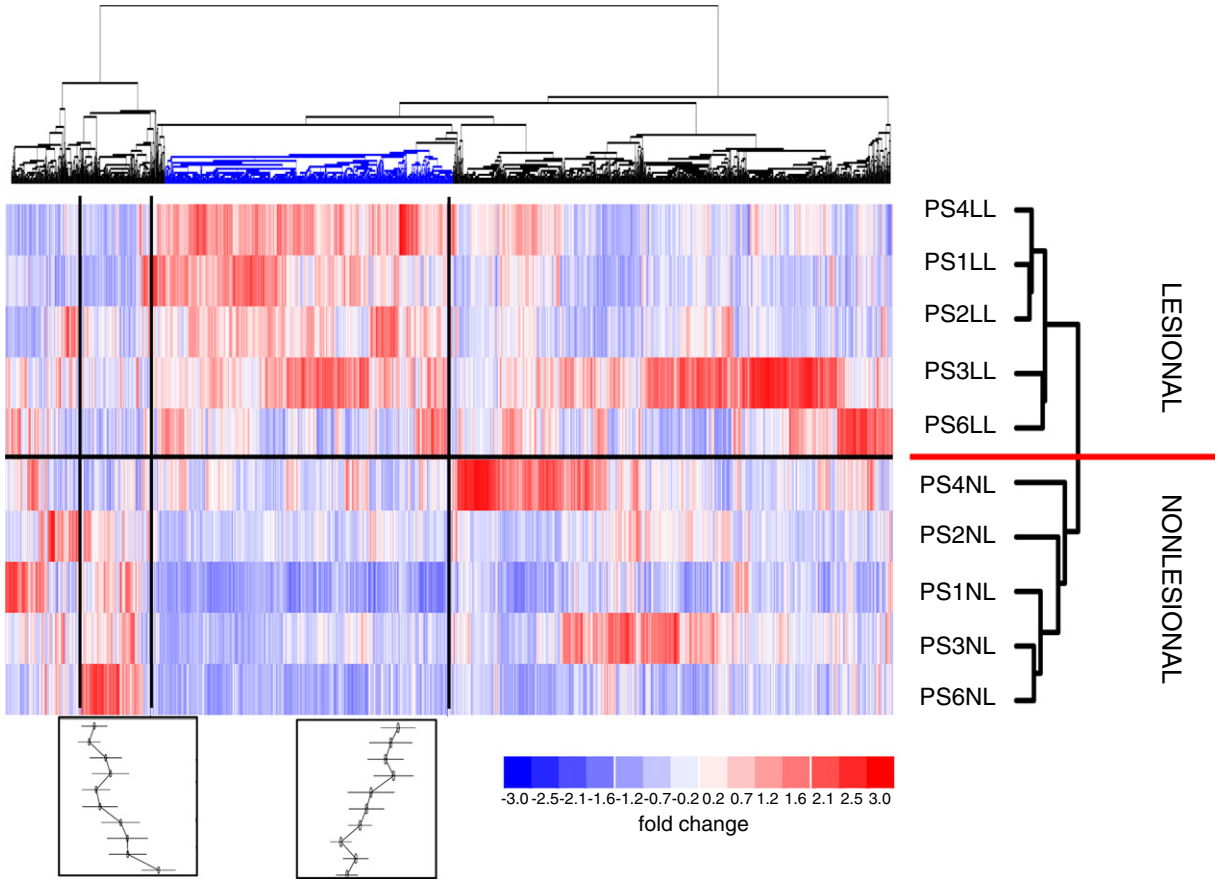


Fig. 1. Unsupervised clustering diagram of the genes expressed in lesional and nonlesional psoriatic skin.Unsupervised clustering clearly differentiates between lesional and nonlesional samples, which are segregated into distinct clusters. The diagram shows 3832 probes passing the filtering criteria of coefficient of variation ≥ 0.3 and present call ≥ 30%, with a clearly distinctive group of 1119 upregulated and 221 downregulated genes in lesional skin. We similarly used unsupervised clustering to evaluate the blood samples obtained from psoriatic and nonpsoriatic subjects. Using the same filtering criteria, we identified 1485 probes corresponding to 1353 unique genes. However, a segregation of cases and controls was not observed with unsupervised clustering. LL, lesional skin; NL, nonlesional skin.

Although we found no immune-related GO terms significantly associated with our list of 535 DEGs, 50 of these genes (9.3%), 44 upregulated and six downregulated, were associated with immune response functions. Nine of these immune-related genes were among the 22 genes upregulated more than five-fold (Fig. 2). Several of the highly upregulated genes, including *SERPINB4, S100A9, S100A7, DEFB4* and *PI3*, are known to be released by keratinocytes in response to both adaptive (IL-17) and innate (IL-1α [12]) immune stimuli. There were seven other DEGs (*S100A8, SPRR2D, CRABP2, NAV3, CXCL1, STAT3* and *TGFB1*) in our list known to be upregulated by IL-17. We also detected nine upregulated genes associated with type 1 interferon signaling (*IFI27, TYMP, IRF7, LAMP3, IFI16, TAP1, MYD88, GCH1* and *RPS6KA1*). We detected seven upregulated genes associated with T cell differentiation and activation (*PPP3CB, DPP4, B2M, TGFB1, SYK, PRKDC* and *FKBP1B*).

2.3. Consensus of DEGs across skin microarray studies

We ran a query in PubMed to find previous reports investigating differential gene expression between lesional and nonlesional psoriatic skin and identified 8 published studies (Oestreicher et al. [6], Zhou et al. [4], Kulski et al. [7], Mee et al. [12], Reischl et al. [11], Yao et al. [10], Gudjonnson et al. [9] and Suarez-Farinas et al. [13]) using microarray methods. Because some of these previous studies were executed on different array platforms, we masked the reported genes that were not included on the Affymetrix HG-U95A arrays used in our study. In the lesional vs. nonlesional psoriatic skin comparisons, the eight studies we included in the comparison group reported a total of 1296 unique genes that were present on the HG-U95A array. In addition, Zhou et al., Kulski et al. and Gudjonnson et al. also compared lesional psoriatic skin to nonpsoriatic control skin and we merged these two lists together to form another group with 858 unique genes.

Fig. 3 shows the overlaps between our list of 535 DEGs (Group I), previously reported DEGs in lesional vs. nonlesional (1296 genes on the HG-U95A array, Group II) and previously reported lesional vs. nonpsoriatic (858 genes, Group III) comparisons. There are 180 genes (33.6%) in our list which overlap with DEGs detected in previous lesional vs. nonlesional comparisons, and 156 genes that were detected in lesional vs. nonpsoriatic comparisons. There were 125 genes consistently identified in all three groups and 12 of these genes, *ALOX12B, IVL, TGM3, SPRR1A, S100A7, TGM1, SPRR2D, SPRR1B, KRT6A, FABP5, CRABP2* and *KRT16* were associated with GO terms related to epidermal development and differentiation. In addition, 12 of these 125 genes were associated with immune response. Also among the overlapping 125 genes, there were 9 genes upregulated by IL-17 (*S100A7, S100A8, S100A9, CRABP2, PI3, SERPINB3*, SPRR2D, *CXCL1* and *DEFB4*).

Among the 1651 unique DEGs reported in the lesional vs. nonlesional comparisons by others and ourselves (union of groups I and II in Fig. 3), there were a total of 570 genes identified in more than one study, 366 as upregulated (142 detected in our study) and 196 as downregulated (33 detected in our study) consistently. Among the 366 replicated genes that were consistently upregulated, 26 were annotated for 'apoptosis', 26 for 'oxidation reduction' and 7 for 'angiogenesis'. We also detected 39 genes related to the type 1 interferon pathway and 5 genes that were regulators of NFκB pathway (*TNFSF10, IL1B, MALT1, UBE2N* and *HMOX1*) in this list. Evaluating the functional annotations of the 196 shared genes that were downregulated, we found significant associations for 10 genes annotated for fatty acid and 29 genes for lipid metabolic processes, and 11 genes for the term oxidation reduction.

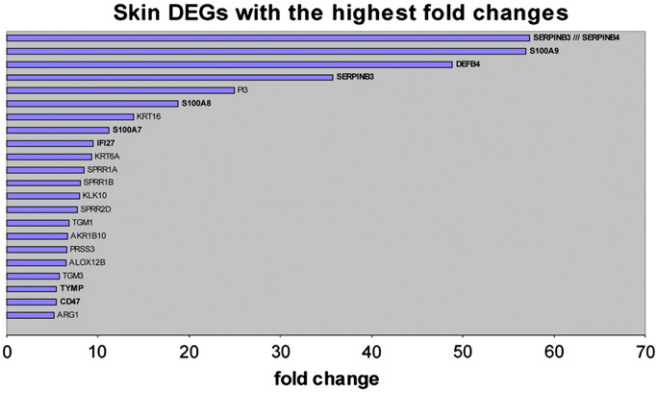


Fig. 2. Skin DEGs with the highest fold changes.Top 22 upregulated genes with fold changes more than 5 in skin. Genes in bold are associated with immune related functions. Complete list of skin DEGs with additional information is in Supplemental File 2.

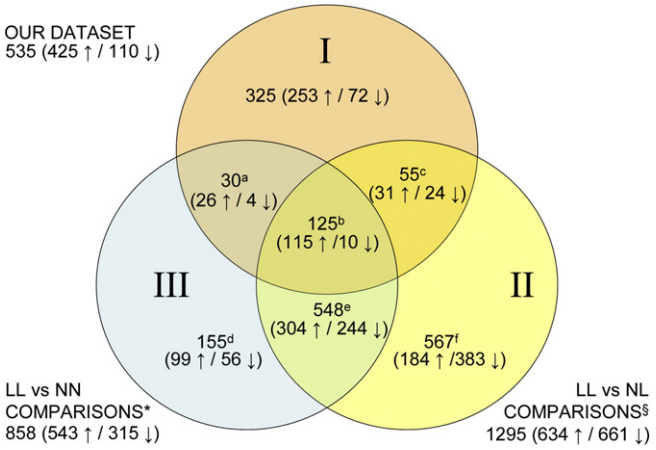


Fig. 3. Overlaps between our dataset and the results of previous studies.Numbers in parenthesis refer to upregulated/downregulated genes in each region. In the overlap regions with genes dysregulated in opposite directions, counts refer to the smaller group number. Group I contains the DEGs between lesional (LL) and nonlesional (NL) psoriatic skin detected in our study at 1% false discovery rate. § Group II contains all DEGs in LL vs NL psoriatic comparisons reported by Oestreicher et al., Zhou et al., Kulski et al., Mee et al., Reischl et al., Yao et al., Gudjonsson et al. and Suarez-Farinas et al. * Group III contains the DEGs between LL vs nonpsoriatic (NN) skin reported by Zhou et al., Kulski et al. and Gudjonsson et al. [a] JUP is upregulated in our dataset and downregulated in Gudjonsson et al.'s LL vs NN comparison. [b] SNX1 is upregulated in our dataset and downregulated in Gudjonsson et al. and Suarez-Farinas et al.'s LL vs NL and Gudjonsson et al.'s LL vs NN comparisons. TRBC1///TRBC2 was downregulated in our dataset and upregulated in Zhou et al.'s LL vs NL and LL vs NN comparisons. SFPQ was upregulated in two LL vs NL (ours and Zhou et al.'s) and two LL vs NN comparisons (Zhou et al. and Kulski et al.) but Kulski et al. also detected the same gene as downregulated in the LL vs NN comparison. [c] KRT2 was upregulated in our dataset and downregulated in Mee et al's, RAB2A was upregulated in our dataset and downregulated in Suarez-Farinas et al.'s, UBE2I was upregulated in our and Oestreicher et al.'s but downregulated in Suarez-Farinas et al.'s dataset. [d] SFN was downregulated in Zhou et al's and upregulated in Gudjonsson et al.'s and VAPA was upregulated in Kulski et al.'s and downregulated in Gudjonsson et al.'s LL vs NN comparisons, [e] SPRR2C was upregulated in Mee et al., Yao et al.'s, Gudjonsson et al. and Suarez-Farinas et al.'s LL vs NL comparisons and Gudjonsson et al. and Kulski et al.'s LL vs NN comparisons but downregulated in Zhou et al.'s LL vs NL comparison. [f] ALG13 was reported as both upregulated and downregulated in Suarez-Farinas et al.'s dataset.

2.4. Gene expression in psoriatic blood

In order to identify the DEGs in blood (between six psoriatic and five nonpsoriatic samples), log-transformed expression indices of the samples were compared with unpaired t-test and FDR 1–5%. We identified 32 DEGs (7 upregulated and 25 downregulated) that distinguished cases from controls at 1% FDR (Table 1), and 153 DEGs (41 upregulated and 112 downregulated) at 5% FDR (Supplemental File 4). Psoriatic blood DEGs did not show significant association with any GO terms at 1% or 5% FDR levels. Nevertheless, there were several DEGs associated with pathophysiologic pathways implicated in psoriasis. Of the 153 DEGs we detected at 5% FDR, 15 (9.8%) genes were associated with immune system functions with 9 upregulated (22.0% of the upregulated genes) and 6 downregulated genes (5.35% of the

Table 1
Dysregulated genes in psoriatic blood. List of 35 genes differentially expressed between psoriatic and nonpsoriatic blood at the 1% FDR level.

Gene symbol	Accession	Gene title	Fold change
Upregulated			
KIAA0040	Z99715	KIAA0040	1.58
TOP3B	D87012	Topoisomerase (DNA) III beta	1.51
NAB1	AF045451	NGFI-A binding protein 1 (EGR1 binding protein 1)	1.42
ATG13	AB014552	Autophagy related 13 homolog	1.38
MCM3AP	AB011144	Minichromosome maintenance complex component 3 associated protein	1.24
GTF2B	M76766	General transcription factor IIB	1.19
LMTK2	AI971726	Lemur tyrosine kinase 2	1.16
Downregulated			
C2CD3	AL080220	C2 calcium-dependent domain containing 3	0.83
SLC6A7	S80071	Solute carrier family 6 (neurotransmitter transporter, L-proline), member 7	0.81
SLC35A3	AB021981	Solute carrier family 35 (UDP-N-acetylglucosamine (UDP-GlcNAc) transporter), member A3	0.8
LYPLA2	AL031295	Lysophospholipase II	0.8
MUC3A	AF007194	Mucin 3A, cell surface associated	0.8
SNX27	AW024812	Sorting nexin family member 27	0.8
FLT3LG	U04806	fms-related tyrosine kinase 3 ligand	0.79
FDXR	J03826	Ferredoxin reductase	0.78
IDH3A	U07681	Isocitrate dehydrogenase 3 (NAD+) alpha	0.78
TOP2A	J04088	Topoisomerase (DNA) II alpha 170 kDa	0.78
FIG4	D87464	FIG4 homolog (*S. cerevisiae*)	0.77
NR0B2	L76571	Nuclear receptor subfamily 0, group B, member 2	0.75
IGFBP1	M74587	Insulin-like growth factor binding protein 1	0.73
NAGPA	AF052111	N-Acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase	0.71
PSKH1	AI767675	Protein serine kinase H1	0.7
STX16	W28230	Syntaxin 16	0.67
EPM2AIP1	AB018309	EPM2A (laforin) interacting protein 1	0.67
NHP2L1	Z83840	NHP2 non-histone chromosome protein 2-like 1 (*S. cerevisiae*)	0.67
PLIN	AB005293	Perilipin	0.66
TPM3	X04201	Tropomyosin 3	0.66
STAT2	U18671	Signal transducer and activator of transcription 2, 113 kDa	0.65
CASP2	U13022	Caspase 2, apoptosis-related cysteine peptidase	0.63
OXCT1	U62961	3-Oxoacid CoA transferase 1	0.63
NCRNA00084	AW006742	Non-protein coding RNA 84	0.56
–	AI817548	–	0.47

downregulated genes). Additionally, there were 9 (5.9%) genes associated with lipid and fatty acid metabolism, 10 (6.5%) with apoptosis and 9 (5.9%) with proteolysis. Nine DEGs detected in psoriatic blood, 1 upregulated and 8 downregulated, were associated with oxidation–reduction functions and 6 genes, 4 of which were upregulated, were annotated for response to oxidative stress (Fig. 4). There were 2 upregulated genes, *CD2* and *TRA@*, associated with the IL17 signaling pathway based on functional annotations listed on the DAVID website (www.david.abcc.ncifcrf.gov).

In the comparison of psoriatic skin and blood DEGs detected in our analyses, we found seven genes that were upregulated (*RANGAP1, RNF40, SNX1, ISG15, MAP2K5, AP3B1* and *DNPEP*) and six genes that were downregulated (*TMEM63A, SLC11A2, SFPQ, MYH11, YIPF6* and *N4BP2L2*) in both datasets at 5% FDR. Two upregulated (*BCR* and *APOM*) and 15 downregulated genes (*ACPP, OXCT1, IDH3A, FIBP, BTF3L1, POP4, TOP2A, RABIF, PPP2R5E, BCL7B, DVL3, FLT3LG, SERBP1, DPT* and *PPFIA1*) in the blood were dysregulated in the opposite direction in the skin.

We also compared our list of blood DEGs to the 25 upregulated and 25 downregulated genes with the highest dysregulation reported by Lee et al. [15] but found no overlap between the two datasets. In order to search for genes commonly dysregulated in systemic comorbidities that have been linked to psoriasis, we further compared our list of DEGs in psoriatic blood with the results of two gene expression studies comparing blood of subjects with and without coronary artery disease. There were no overlaps between our list of blood DEGs at 5% FDR and that reported by Sinnaeve et al. [16]. Seven genes from the list of DEGs reported by Wingrove et al. [17] were included in our set. However, only *LAIR1* was upregulated in both sets and the remaining six genes (*SASH1, CDK11A, ACPP, PPFIA1, CYP4F2* and *DEC1*) were downregulated in psoriasis and upregulated in coronary artery stenosis.

2.5. Mapping of DEGs to the genome

We evaluated the chromosomal distribution of psoriasis skin and blood DEGs across the genome and compared their localizations to the 12 PSORS loci reported by linkage and association studies [18–27]. At the 1% and 5% FDR levels, 64 of 535 (12.0%) and 159 of 1529 (11.8%) of the skin DEGs correspond to one of these 12 loci, respectively. In addition, 18 of the 153 (10.4%) DEGs we identified at 5% FDR in psoriatic blood also map to PSORS loci. The highest number of DEGs in the skin are detected at 1q21 (PSORS4) and 19p13 (PSORS6), with 15 and 14 genes at 1% FDR, and 25 and 32 genes at 5% FDR levels, respectively. At the 5% FDR level, we also detected 22 and 20 DEGs at PSORS8 and PSORS11, respectively. The complete list of DEGs from our study that maps to PSORS loci is presented in Supplemental File 5. We also evaluated the overlap of the 570 genes shared across skin microarray studies to the PSORS loci and found 67 DEGs mapping to the 12 susceptibility loci (Supplemental File 6). PSORS4 was the loci where most of the shared genes were located with 11 genes mapping to this region, including members of the S100 and SPRR families *IVL* and *CRABP2*, being reported by more than 5 studies.

Next, we used the 'genome' tool in dChip to identify the genomic regions where DEGs cluster more frequently than would be expected to occur by chance. Using a threshold of $p \leq 0.0001$, we first mapped the skin DEGs from our study at 1% FDR level and identified four transcriptional "hot spots": 1q21.1-25.3 (which contains PSORS4), 6p25.3-p24.3, 11q12.1-q13.2 and 16p11.2. When we repeated the analysis at 5% FDR level, we identified 20 "hot spots", 2 of which overlap with the confirmed susceptibility loci PSORS4 and PSORS11, and 4 with previously suggested loci at 3p21-23, 9q34, 12q13.2, and 13q21-32 [19,22,28]. We also detected 14 novel regions where DEGs are significantly over-represented. DEGs in these transcriptional "hot spot" regions are listed in Supplemental File 7.

We detected two regions of significant accumulation for blood DEGs at 6p25.1-p25.2 and 11q12.2-q13.4. These two regions overlapped with two of the four "hot spots" identified for skin DEGs at 1% FDR. Fig. 5 shows the distribution of all "hot spots" for blood and skin DEGs detected in our analysis along with their overlaps with one another and with reported disease susceptibility loci.

For the 570 genes replicated across the eight microarray studies comparing lesional and nonlesional skin, at the $p \leq 0.0001$ threshold we identified 6 "hot spots" at 1q21.1-q24.2, 4q13.2-q21.1, 10q22.3-q23.33, 11q12.1-q13.2, 18q21.33 and 19q13.32-q13.33 (Fig. 6). These "hot spots" overlap with one confirmed (PSORS4 at 1q21) and two reported (4q13 [29] and 4q21 [19]) susceptibility loci. The replicated genes are also significantly frequent at a region overlapping both our skin and blood data at 11q12.1-q13.2.

3. Discussion

In this study, we compared global gene expression between lesional and nonlesional psoriatic skin samples and confirmed the distinction between the two skin types. Unsupervised clustering clearly differentiated cases from controls and the upregulated genes driving this difference were mainly related to functions associated

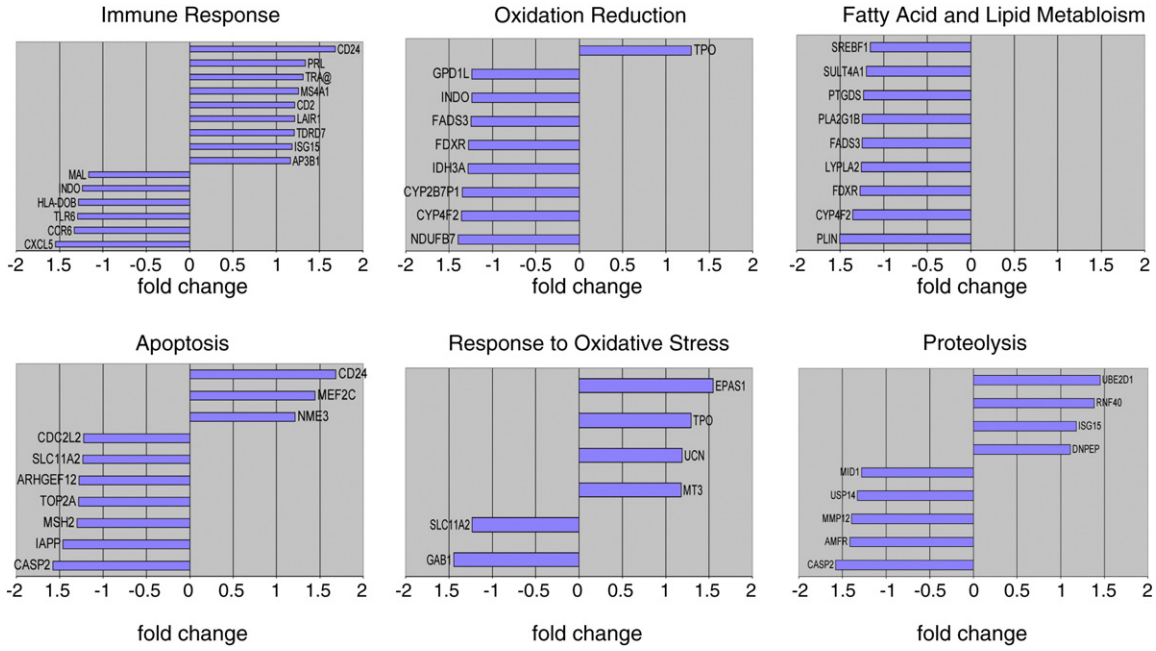
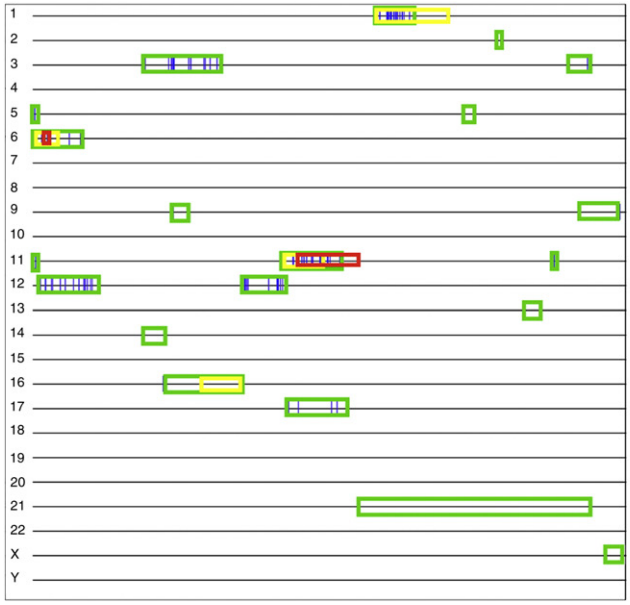


Fig. 4. Psoriatic blood DEGs associated with pathophysiologic pathways implicated in psoriasis.

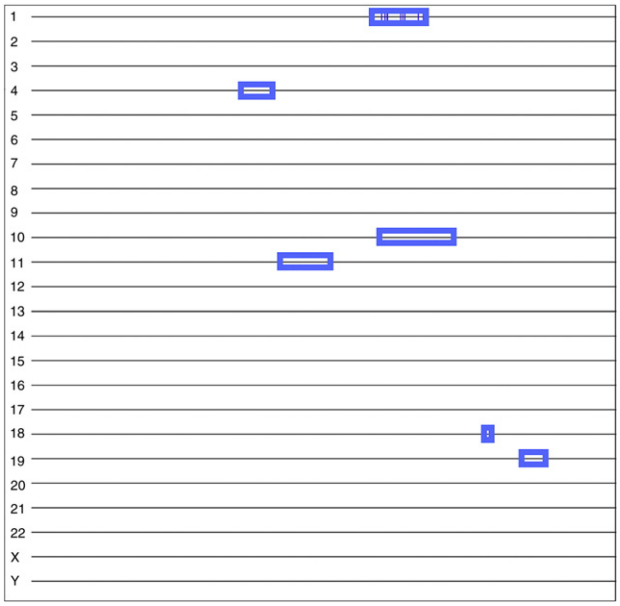
with epidermal differentiation, cell cycle and metabolism, which are consistent with the pathophysiologic changes related to the hypermetabolic epidermis in the psoriatic plaque. The list of 535 DEGs detected at 1% FDR was also significantly associated with epidermal differentiation, cell cycle and metabolism functions, as seen for the upregulated genes in unsupervised clustering analysis.

Functional relevance of reported DEGs to disease processes may be strengthened by consensus across similar studies. Previous comparisons of microarray analyses in psoriasis have arrived at contradicting conclusions. While Romanowska [30] reported a high concordance rate with the results of Zhou et al. [4], Kulski et al. [7], who compared their results with three earlier studies [5,6,31], claimed concordance to be 'at best only moderate'. Gudjonsson et al. [9] detected a 'limited' overlap in the comparison of their dataset with two earlier lesional vs nonlesional psoriatic skin analyses [4,6], but reported a higher concordance with a more recent study by Yao et al. [10] performed on the same microarray platform. In a recent report, Suarez-Farinas et al. [13] compared their dataset to the results of 3 previous microarray studies [4,9,10] with the gene set enrichment analysis method. They demonstrated a higher degree of consensus that was not evident in the comparisons of the reported DEG lists by evaluating each set of DEGs ranked according to fold changes to the complete probe list in their study, rather than only the identified set of DEGs. We also observed a moderate level of concordance, both between our dataset and the results of previous studies, and across the nine datasets included in our cross-comparison. There were 180 (33.6%) DEGs among the 535 we detected that were reported by at least one



SKIN		BLOOD		
Hotspots	DEGs	Hotspots	DEGs	Overlapping Susceptibility Loci
1q21.1-q23.3	35			PSORS4
2q32.2-q32.3	7			
3p22.2-p14.1	26			3p21-23
3q26.32-q27.2	15			
5p15.33	4			
5q31.1	8			PSORS11
6p25.3-p23	30	6p25.2-p25.1	4	
9p13.3-p13.2	7			
9q34.11-q34.3	12			9q34
11p15.5	5			
11q12.1-q13.3	33	11q12.2-q13.4	8	
11q23.3	4			
12p13.3-p12.3	26			
12q13.11-q14.1	27			12q13.2
13q32.1-q32.2	6			13q21-32
14q11.2-q12	16			
16p12.3-p11.2	20			
17q12-q21.31	22			
21q21.3-q22.3	17			
Xq28	9			

Fig. 5. Genomic "hot spots" for the skin and blood DEGs.With the use of the 'genome' tool in dChip, it is possible to identify the genomic regions where DEGs are located more frequently than it would occur by chance. The figure shows the "hot spots" for DEGs detected in the blood at 5% FDR (l, skin at 1% (yellow) and skin at 5% FDR (green). The table presents the number of skin and blood DEGs detected at 5% FDR in the "hot spots" and the overlapping susceptibility loci reported previously for that region. Skin "hot spots" in bold overlap with "hot spots" identified for the skin at 1% FDR. Complete list of genes mapping to these "hot spots" are listed in Supplemental File 7.



Hotspots	Number of DEGs	Genes	Overlapping susceptibility loci
1q21.1-q24.2	27	S100A family, SPRR family, CD2, IVL	PSORS4
4q13.3-q21.1	9	IL8, CXCL1, CXCL9, CXCL10	4q13 and 4q21
10q22.3-q23.33	13		
11q12.1-q13.2*	12	FADS1, FADS2, SCGB family, TCN1	
18q21.33**	4	SERPINB3, SERPINB4, SERPIN8	
19q13.32-q13.33**	8	KLK6, KLK8 /// KLK9, KLK10, KLK13	

Fig. 6. Genomic "hot spots" for the skin DEGs replicated in microarray studies.Chromosomal "hot spots" of the top 570 DEGs are noted as bolded blue vertical bars. p-Values were calculated for all stretches containing < or = 20 differentially expressed genes to assess the significance of "gene proximity". 335 of the 570 are located significant stretches (of < or = 20 genes) with $p < 0.0001$ were considered "hot spots" of statistical significance. * 11q12.1-q13.2 overlaps with a region we detected as a hot spot for both our skin and blood DEGs in our analysis. ** 18q21 and 19q13 were two regions where Gudjonsson et al. also reported an accumulation of highly overexpressed genes in his dataset, which were very similar to the genes we found in these two regions.

previous study. In order to standardize the results of studies performed on different microarray platforms, we filtered the reported datasets to include only those genes included on the HU-G95A array. Of the 1651 DEGs reported by nine lesional vs. nonlesional comparisons, only 34.5% were replicated in at least one other study.

A lack of consensus between microarray datasets from different studies on given disorders has been previously discussed by our group and others [13,32]. In addition to the random noise associated with high throughput analysis and differences in experimental and technical methods used [9,33], the limited consensus in psoriasis may also relate to heterogeneity of subjects across studies. A recent study employing RNA-seq also demonstrates the lack of consensus, with only 44% of their genes overlapping with previous microarray studies [34]. Differences in methods, which may be exemplified by demographic differences between the subjects and the criteria used across microarray studies to define and report DEGs, also includes the microarray platforms used, which may vary in the number and actual location of the probes used for the same regions of the transcriptome. The list of 570 genes that are replicated across the nine studies included in our analysis may provide a distilled set of core genes that are particularly relevant to lesional pathology.

Although our dataset confirmed significant functional pathway associations for epidermal hyperproliferation, we found no significant association with immune response functions and the numbers of genes related to the recently emphasized pathways in psoriasis, including Th1, Th17 and dendritic cells along with IL23, NFκB and type 1 interferon signaling [1–3,10,22,35,36], were somewhat limited. One possible reason that would prevent the detection of significant associations with these pathways may be lack or absence of probes for the related genes on the microarray platform [13]. In a review of microarray studies, Kunz noted the absence of IL-17, IL-22 and IL-23 in psoriasis reports and mentioned the low level of these cytokines they detected in rheumatoid arthritis with the microarray method [37]. Lack of significant associations with these immune related pathways may also be due to either a weak immune response in the lesional psoriatic skin, or, possibly, an already active immune response in the nonlesional skin, setting a high baseline that may have masked the extent of the response in the lesional samples. In fact, when we analyzed the group of 858 unique genes reported by Zhou et al., Kulski et al. and Gudjonsson et al. in their lesional psoriatic vs. nonpsoriatic skin comparisons (Group 3 in Fig. 4), we did see significant associations with immune response related GO terms 'regulation of immune system process', 'immune response' and 'immune system process'. Among these 858 genes, 137 were associated with immune system processes, indicating that there indeed is an active immune response in the psoriatic plaque that is not evident in the lesional vs. nonlesional comparison. When we further analyzed the group of 228 unique genes reported by the same three studies to be differentially expressed in the nonlesional psoriatic vs. nonpsoriatic skin comparison in order to see the immune response functions, we found significant associations with GO terms 'response to other organism' and 'response to biotic stimulus', related to 14 genes (*DEFB4, DNAJB6, HSPA8, PPBP, CREB3L1, IFI44, IFNGR1, MX1, MYD88, PLSCR1, S100A12, S100A7, STAT1* and *TRIM22*).

These associations indicate an already evident immune activity in nonlesional psoriatic tissue. Gudjonsson et al. have recently reported the upregulation of genes associated with innate immunity, including *DEFB4, RNASE7* and *IL36G*, in the nonlesional psoriatic skin compared to nonpsoriatic skin [38] which appears to represent a 'pre-psoriatic' or 'stand-by' state. Expression of angiogenetic markers, vascular volume and adhesion molecules are all increased in phenotypically normal psoriatic skin, consistent with the observation that the earliest recognizable change in psoriasis is the perivascular accumulation of mononuclear leucocytes [39]. If the immune response activity is already set at a high level in the nonlesional skin, dysregulation of a limited number genes may be suffice to initiate plaque formation. The list of commonly replicated genes in lesional vs. nonlesional skin comparisons may suggest new targets for treatment that would prevent the conversion from 'pre-psoriatic' to active phase.

We also compared the blood from psoriatic subjects to the blood from nonpsoriatics to detect the differences in gene expression associated with psoriasis at the systemic level. The number of DEGs and the range of associated fold changes we detected in the blood were limited compared to the differences observed between lesional and nonlesional skin. Comparison of the gene expression between psoriatic and healthy blood reported by Lee et al. [15] also reported a relatively short list of DEGs between psoriatic and normal blood, with a narrow range of fold

changes between 3.8 and −4.4. Despite these similarities, there were no overlaps between our set of blood DEGs and list of genes reported by Lee et al. As might be expected, the transcriptional changes observed in lesional psoriatic skin were not directly reflected in psoriatic blood. Yet, there were several DEGs in the blood related to the dysregulated pathways implicated in psoriasis pathophysiology including immune function, apoptosis, proteolysis, and lipid and fatty acid metabolism. We also detected eight downregulated genes associated with oxidation reduction processes, which may indicate an increased oxidative stress operative at the systemic level. Reactive oxygen species interfere with various signaling pathways involved in psoriasis, including MAPK/AP-1, NFκB and JAK/STAT [40].

Although systemic comorbidities, like cardiovascular disease, associated with psoriasis may suggest systemic as well as lesional psoriatic gene expression signatures, our results did not reveal broad-scale inflammatory changes in the blood. The overlap between the psoriatic blood DEGs reported here and the results of two gene expression studies comparing patients with coronary artery disease to controls [16,17] was also poor. Nonetheless, cardiovascular risk in psoriasis was initially documented in hospitalized subjects [41–43] and the subsequent community based studies reported a stronger association with severe psoriasis [44–46]. It is possible that dysregulation in the peripheral blood of severe cases, in contrast to the mild–moderate cases included in our analysis, may involve a different set of genes associated with systemic response. Larger studies enrolling subjects in defined subgroups that would enable further stratification based on disease severity, along with age of onset, morphology and response to therapy which aim to investigate the differential gene expression not only in psoriatic skin but also in the blood will be important to help illuminate the molecular genetic basis of the extensive disease heterogeneity and systemic involvement seen in psoriasis.

For the past two decades, familial linkage analysis and genome wide association studies have suggested nearly forty psoriasis susceptibility loci [19,21,23,24,29]. Twelve of these (PSORS1-12) have been confirmed by multiple studies in different populations. However, no susceptibility gene has been definitively confirmed within these regions, except for very strong evidence provided by Nair et al. for HLA-Cw6 at PSORS1 locus [47]. Focus on DEGs reported by microarray analysis may provide a new strategy to pinpoint the susceptibility genes in the broader regions detected by genome studies. In fact, in a recent report Chen et al. demonstrated differentially expressed genes to be more likely to harbor disease-related mutations [14]. Thus, merging of transcriptional and genetic datasets may facilitate the identification of the comprehensive set of psoriasis susceptibility genes, as transcriptional changes may, at least in some cases, reflect the functional results of genetic variations associated with the disease. For this purpose, we examined the list of DEGs corresponding to reported susceptibility loci. Genes replicated across the microarray studies that map to the PSORS loci may be especially notable as the chances of a gene to be associated with disease increased with the number of studies reporting the gene [14]. This data may provide a useful guide to pinpoint psoriasis-associated genes within these regions.

We found several overlaps between previously reported susceptibility loci and genomic or chromosomal locations of transcriptional "hot spots" we detected in both our set of DEGs and the set of DEGs that are replicated across studies. At chromosomal region 1q21, where both PSORS4 and the epidermal differentiation complex (EDC) are mapped [48], loricrin (LOR), involucrin (IVL), SPRR family and calcium binding S100 family genes have been consistently replicated DEGs. While associations between psoriasis and a number of genes at this region have been reported, there has been no conclusive evidence in any case. DEGs overlapping to this region may suggest possible new linkages at this locus. Our set of skin DEGs also showed significant accumulation at 5q31.1, overlapping with PSORS11.

One of the significant regions for both our skin and blood datasets was also significant for the set of genes replicated across microarray studies. At chromosomal region 11q12.1-q13.2, we detected immune related genes *TCIRG1*, *OTUB1*, *RBM4* and *UBE2L6* along with *RELA*, a component of the NFκB pathway in our skin dataset. Replicated genes in this region also included *FADS1, FADS2, CST6, GAL* and *CCND1*. This region was also reported to contain a balanced translocation in two of the 477 psoriatic subjects in a cytogenetic analysis [49], Examination of transcriptionally dysregulated genes within reported susceptibility loci offers a new strategy to narrow the search for candidates in future genetic analyses.

In summary, our data extend the support for major pathway disturbances related to epidermal hyperproliferation in the lesional psoriatic skin. The set of genes replicated across studies presents a comprehensive picture of dysregulated pathways in psoriasis that may not be evident in the analyses of individual datasets. We also elucidate the gene expression profiles in the blood of mild–moderate cases, which contain several genes related to previously implicated pathways in psoriasis. Investigation of gene expression alterations in the blood of severe cases may further illuminate molecular pathways related to the systemic effects of psoriasis. Transcriptional analysis can be expected to aid in the development of tools for the molecular classification of disease with diagnostic and possibly prognostic value. Furthermore, the pool of psoriasis associated skin and blood DEGs should provide guidance to focus future candidate gene screenings for the precise localization of disease susceptibility loci.

4. Methods

4.1. Samples

Nine subjects (6 females, 3 males), aged between 32 and 77 years, with a clinical diagnosis of psoriasis vulgaris were recruited from the Dermatology Outpatient Clinic of New York Presbyterian Hospital, Cornell University (IRB # 0998-398). All patients had mild–moderate chronic plaque psoriasis, did not have a history of systemic treatment for psoriasis, were free of phototherapy at least for one month prior to the biopsy date and had PASI scores < 12. After obtaining informed consent, a total of four 6 mm punch biopsy samples, two from psoriatic lesions and two from site-matched nonlesional skin were taken from each of the nine subjects. All skin samples were snap frozen in liquid nitrogen. Additionally, blood samples from 8 psoriasis (3 females, 5 males, aged between 27 and 72 years) and 5 nonpsoriatic control subjects (2 females, 3 males, aged between 23 and 63 years) were also collected and frozen at −80 °C.

4.2. RNA extraction and biotinylated cRNA preparation

Peripheral blood mononuclear cells were isolated from blood samples with the use of Ficoll gradients (Amersham Biosciences, Piscataway, NJ). Total RNA extraction from the tissue and blood samples was performed using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA). RNeasy Mini kit (QIAGEN Inc, Valencia, CA) was used for RNA purification. Microarray assays were processed according to the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA, http://www.affymetrix.com). Using 16 μg of total RNA, double stranded cDNA was synthesized to be purified and concentrated by ethanol precipitation. Biotinylated cRNA was produced by in vitro transcription and fragmented to 50–200 nucleotides. The labeled cRNA was hybridized for 16 h at 45 °C to Affymetrix HG-U95A or HG-U95Av2 microarrays, which contain approximately 12600 probe sets. After washing the microarrays, streptavidin-phycoerythrin staining was performed. The staining was amplified using biotinylated anti-streptavidin and streptavidin-phycoerythrin for scanning (HP GeneArray Scanner, Affymetrix).

4.3. Image acquisition, low-level analysis

Microarray Suite v5.0 (Affymetrix Inc., Santa Clara, CA) was used to generate DAT and CEL files, which were imported to dChip using a probe set mask file to combine data from HG-U95A and HG-U95Av2 arrays. After applying the probe set mask, analyses were carried out using 12577 probe sets corresponding to 9748 unique genes. CEL image files were checked for hybridization artifacts in dChip before and after normalization and model based expression index (MBEI) calculation. All arrays were normalized using the invariant set method. MBEI were calculated in dChip by fitting the model to the PM–MM differences across all arrays. Arrays flagged as outliers were removed from the dataset and the normalization was repeated for the remaining arrays until achieving a sample with no outliers. Final dataset comprised a total of 21 arrays, which include 10 skin samples (5 lesional and 5 nonlesional from 5 psoriatic patients) and 11 blood samples (6 psoriatic and 5 from nonpsoriatic subjects).

4.4. Unsupervised hierarchical cluster analysis and differential gene expression

Probe sets were filtered to identify the most informative set to be used in unsupervised cluster analysis. Probes that were called present in at least 30% of the arrays and those with a coefficient of variation greater than 0.3 were included in unsupervised clustering. There were 3832 probes fulfilling these criteria, which corresponded to 3412 unique genes. A two-way cluster analysis was performed on the probe sets that passed the filter in dChip using centroid linkage and the 1-r metric.

Differentially expressed probe sets between cases (lesional skin and psoriatic blood) and controls (nonlesional skin and nonpsoriatic blood) were defined according to i) having a present call in at least 2 arrays among either cases or controls and ii) having a $p \leq 0.01$ in the two tailed t-test (paired for skin and unpaired for blood samples) between log2 transformed MBEI from case and control arrays AND passing the designated Benjamini–Hochberg critical values.

Enrichment of gene ontology (GO) terms was tested for significance in dChip ($p \leq 0.001$), which uses the binomial approximation to the hypergeometric distribution to calculate p values. The degree of enrichment for a GO term was assessed by calculating the odds ratio for enrichment of a given GO term in a specified gene list. DEGS were annotated for immune related functions manually using the online sources at www.pubmed.org Entrez Gene pages and using the GO terms provided by dChip and the Database for Annotation, Visualization and Integrated Discovery (DAVID) website (www.david.abcc.ncifcrf.gov) [50].

4.5. Quantitative real time reverse transcriptase polymerase chain reaction (QRT-PCR) validation

Total RNA from skin samples, where sufficient amounts of total RNA were available after microarray analysis, was used for QRT-PCR analysis to confirm the validity of microarray analysis for 8 randomly selected genes in the skin (*TGM1, POLE3, IVL, FABP5, IL1RN, LYN, GARS* and *ALOX12B*). Primers for these genes were designed using Primer Express software.18S rRNA was used as an internal control. The resulting fold changes were compared to those obtained by microarray analysis.

4.6. Query for microarray studies

We ran a query in PubMed using the search terms 'psoriasis AND microarray' and 'psoriasis AND gene expression array' to find studies performed on psoriatic subjects using microarray methods in order to form a comparison group for our data. We excluded the studies that did not involve a lesional vs nonlesional comparison or provide a global, as opposed to pathway specific, list of dysregulated genes. Of the nine studies we detected, we further excluded the study by Bowcock et al. [5] since the same samples were also used in a later analysis by Zhou et al. [4], which is included in our comparison group. Results of the analysis by Romanowska et al. [30] were not included because the presented list contained only those genes also reported by Zhou et al. Results of the remaining 8 studies by Oestreicher et al. [6], Zhou et al. [4], Kulski et al. [7], Mee et al. [12], Reischl et al. [11], Yao et al. [10], Gudjonsson et al. [9] and Suarez-Farinas et al. [13] were merged together to form a comparison group. DEGs detected in involved vs. nonpsoriatic skin comparisons reported by three of these studies, by Zhou et al., Kulski et al. and Gudjonsson et al., were also merged to form a third group of genes. The reported lists of genes, and the full lists whenever provided by the contacted authors, were filtered to include only those genes present on the HU-G95A array. Merging of the lists was accomplished by identifying all probes assigned to the Entrez Gene ID of any reported probe. Unigene accession numbers were used to identify the associated probes where reported probe did not have an assigned Entrez Gene ID.

We also searched for microarray studies comparing psoriatic and nonpsoriatic blood with the same criteria. In addition to a number of investigations analyzing the treatment effects on gene expression in psoriatic blood before and after treatment, there was only one microarray study directly comparing gene expression between psoriatic and nonpsoriatic blood. We used the list of most strongly dysregulated genes provided in this publication to compare our blood data, as we could not access the full list of dysregulated genes.

5. Competing interests

The authors declare that they have no competing interests.

6. Authors' contributions

AC and MI participated in the analysis of the results and drafting of the manuscript. JRS participated in the analysis of the results and drafting of the manuscript. AAS designed the study, coordinated the microarray procedures and helped to draft the manuscript. All authors read and approved the final manuscript.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ygeno.2012.05.004.

Acknowledgments

Authors would like to thank the staff and healthcare personnel at New York Presbyterian Hospital, Cornell University for their help in patient recruitment and sample collection.

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